Study Title:

Request for Waivers of Non-Target Aquatic Organism Studies for Silver Dihydrogen Citrate Products

Data Requirements:
850.1350: Mysid Chronic Toxicity
850.1400: Fish Early-Life Stage Toxicity
850.1735: Whole Sediment: Acute Freshwater Invertebrate Toxicity
850.1740: Whole Sediment: Acute Marine Invertebrate Toxicity
850.1850: Aquatic Food Chain Transfer

Study Completion Date:
September 26, 2020

Author:
Erik Janus
Steptoe & Johnson, LLP

Submitter:
ETI H2O, Inc.
771 Jamacha Road #512
El Cajon, CA 92019

Report ID:
ETI-2020.03

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this report on the basis of its falling within the scope of FIFRA Section 10(d)(1)(A), (B), or C). The submission of this report in compliance with FIFRA does not constitute a waiver of any right to confidentiality that may exist with respect to any other statute in the U.S., or in any other country.

Company: ETI H2O, Inc.

Company Agent/Submitter: ______________________________
Dolana Blount
VP, Product Development/Regulatory Affairs

Date: 09.26.2020

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This volume provides the rationale for waiver requests. The following exposure and risk assessment information is not subject to the principles of the U.S. Environmental Protection Agency's Good Laboratory Practice (GLP) Standards as set forth in 40 CFR Part 160.

Submitter: ______________________________

Dolana Blount
VP, Product Development/Regulatory Affairs

Date: 09.26.2020

Request for Waiver of Non-Target Aquatic Organism Studies
for Silver Dihydrogen Citrate Products

Waivers are requested from the requirements to conduct non-target aquatic organism toxicity studies OCSPP 850.1350, 850.1400, 850.1735, 850.1740 and 850.1850 as requested by the July 6, 2012 Generic Data Call-In, GDCI-072500-1194 (2012 GDCI) and reinstituted in September 2019. Per the letter from EPA on September 3, 2019, all the ETI H2O silver dihydrogen citrate (SDC) products have been reclassified from Silver (elemental), PC Code 072501, to Silver ion, PC Code 072500.[1]

Introduction & Background

In the 2019 letter, EPA has reinstituted its previous request that that several non-target aquatic organism toxicity studies be submitted on ETI H2O's silver-containing antimicrobial products.

Per the 2012 GDCI and 2019 letter, the Agency has requested the following studies:

1. OCSPP 850.1350: Mysid Chronic Toxicity
2. OCSPP 850.1400: Fish Early-Life Stage Toxicity
3. OCSPP 850.1735: Whole Sediment: Acute Freshwater Invertebrate Toxicity
4. OCSPP 850.1740: Whole Sediment: Acute Marine Invertebrate Toxicity
5. OCSPP 850.1850: Aquatic Food Chain Transfer

The footnote for 850.1350 and 850.1400 specify that these data are "required on estuarine species if the product is: (i) intended for direct application to the estuarine or marine environment; (ii) expected to enter this environment in significant concentrations because of its expected use or mobility patterns, and (iii) if the acute LC50 or EC50 < 1 mg/l; or (iv) if the estimated environmental concentration in water is equal to or greater than 0.01 of the acute EC50 or LC50 and any of the following conditions exist: (a) studies of other organisms indicate the reproductive physiology of fish and/or invertebrates may be affected; (b) physicochemical properties indicate bioaccumulation of the pesticide; (c) the pesticide is persistent in water (e.g., half-life in water greater than 4 days)." (2012 GDCI, Footnote 20).

The footnote for 850.1735 and 850.1740 specify that testing is "required if the soil partition coefficient (Kd) is equal to or greater than 50 and the pesticide is persistent (e.g., half-life in sediment is equal to or less than 10 days) in either the aerobic soil or aquatic metabolism studies. Registrants should consult with the Agency on appropriate test protocols." (2012 GDCI, Footnote 2).

For 850.1850, the footnote specifies that these are "required based on the results of fish or aquatic nontarget organisms accumulation studies (guidelines 850.1730 and 850.1950). (Footnote 13).

---

[1] See letter from EPA to Steptoe & Johnson LLP, dated September 3, 2019. Subject: Amendment to the Generic Data Call-In Notices for Products Containing Silver Particles.

ETI H2O, a division of PURE Bioscience produces four (4) silver dihydrogen citrate (SDC) containing products.[2] The MUP concentrate (Product 1 below) contains 2400 ppm or 0.240% silver ion and 20.66% citric acid with the remainder consisting of deionized water, produced in an integrated system. Silver *per se* is not present in an isolated form; it exists only as an ion stabilized by citric acid. There is no particulate matter in these products, nor are there any nano-scale particles of silver.[3]

The four registered products are:

1. Axenohl (Reg. No. 72977-1) which is 2400 ppm or 0.240% silver ion and 20.66% citric acid. This is the manufacturing use product (MUP).
2. Axen30 (Reg. No. 72977-3) which is 30 ppm or 0.003% silver ion, 4.8% citric acid
3. Axen50 (Reg. No. 72977-4) which is 50 ppm or 0.005% silver ion, 5% citric acid.
4. SDC3A (Reg. No. 72977-5) which is 30 ppm or 0.003% silver ion, 4.8% citric acid.

These products are: a MUP for making antimicrobial end use products; and, ready to use sprays and solutions for indoor use. Application of the product is achieved via non-aerosol-generating-method (i.e. spray trigger, pump spray, etc.). The directions for use specify that no potable water rinse is needed following application and surfaces can either be wiped with a clean towel or allowed to air dry after sufficient contact time.[4]

The overall weight of evidence rationale for the waivers is as follows:

1. The labels for all the SDC products specify indoor uses only. Instructions for use include application to indoor hard surfaces via a non-aerosol-generating-method and do not require wiping the surface or a potable water rinse. Thus, there is no viable mobility pathway for residual silver to travel into the environment, much less come into contact with a non-target organism, aquatic or otherwise.

2. EPA has previously agreed with this assertion in granting SDC products (in a manner that is separate from silver salts and silver zeolites) a tolerance exemption for use on food-contact surfaces. In this approval, EPA recognized that "[t]he uses identified as indoor hard surface applications will result in minimal, if any, runoff of silver into the surface water. The use of silver as a food contact surface sanitizer will result in minimal, if any, runoff of silver into the surface water. This use will result in an insignificant contribution to drinking water exposures." (74 FR 27447, at 27451).

3. This "minimal runoff" actually poses very low exposure potential as the vast majority of silver ions will be removed through the wastewater treatment process. The small amounts of silver ion that may pass through a WWTP unchanged will be removed from the post-treatment

2 See 74 FR 27745, July 10, 2009, where the Agency identifies this product as "Silver ions resulting from the use of electrolytically-generated silver ions stabilized in citric acid as silver dihydrogen citrate."

3 The original DCI requirements from 2012 were placed on hold in 2015 pending an EPA review intended to determine whether existing registered products should be reclassified as "nanosilver." See letter from EPA from September 3, 2019.

4 For example, see Directions for Use for Axen 50 (EPA Registration # 72977-4) label dated August 6, 2009.

water column rapidly and completely, with most of the now non-reactive silver being incorporated into sediment near where it was originally deposited.

4. The toxicity of silver to aquatic organisms of regulatory concern has already been well characterized by both past EPA documents as well as reports found in the open literature.

5. Given the amount of data available on silver ecotoxicity, additional data would not provide any additional informative value to ecological risk assessment (as wildlife toxicity values for silver already exist).

Regulatory Basis For Requested Waivers

At 40 CFR 158.45, the Agency identifies that waivers for data requirements can be considered:

> a) The data requirements specified in this part as applicable to a category of products will not always be appropriate for every product in that category. Some products may have unusual physical, chemical, or biological properties or atypical use patterns which would make particular data requirements inappropriate, either because it would not be possible to generate the required data or because the data would not be useful in the Agency's evaluation of the risks or benefits of the product. The Agency will waive data requirements it finds are inappropriate, but will ensure that sufficient data are available to make the determinations required by the applicable statutory standards.

The Agency's strategic objective for toxicity testing, as identified at https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/strategic-vision-adopting-21st-century-science, clearly identifies the Agency's objective of "Refining and reducing animal testing by maximizing information obtained from animal studies, and focusing on effects of concern" and moving from testing for 'completeness' to carefully focused animal testing where concerns exist, using ... hazard-based hypotheses about the plausible toxicological potential of a pesticide or group of pesticides based on their physical-chemical properties." This is consistent with the National Academies of Sciences recommendations to EPA and is an essential component of the Agency's objectives.[5]

No viable mobility pathway exists for indoor use of SDC products

ETI H2O's products are a MUP for making antimicrobial end-use products and ready-to-use sprays and solutions. All of the products are registered only for indoor use and do not have any outdoor use patterns. Application of the product is achieved via non-aerosol-generating-method (i.e. spray trigger, pump spray, etc.) followed by wiping or air drying. In other words, the label directions do not require the product be wiped up after application and there is no need for a potable water rinse following application. In fact, for certain use sites (such as meat, poultry and

[5] The recommendations provided to EPA can be found in the 2007 National Academies of Science/National Research Council report, "Toxicity Testing in the 21st Century: A Vision and a Strategy." Information regarding the implementation of these recommendations can be found here: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/strategic-vision-adopting-21st-century-science.

dairy processing plants), the use of a potable water rinse following application is not allowed (for example, see the Axen 50 label).

In fact, the EPA agrees with this assertion, according to the justification it provided in granting an exemption from a tolerance for food-contact uses of SDC products. In addition to FIFRA registration, the SDC products have received an exemption from tolerance (74 FR 27447, June 10, 2009). This was granted *specifically* to silver ions stabilized with citric acid as SDC, and not extended to "any other silver-containing compounds whether they are other silver salts, complexes with inorganic polymers such as zeolites, or metallic silver in any form or dimension including nanoscale."

Beyond reviewing the low mammalian toxicity of SDC, in granting the tolerance exemption, EPA recognized that, "[T]he uses identified as indoor hard surface applications will result in minimal, if any, runoff of silver into the surface water. The use of silver as a food contact surface sanitizer will result in minimal, if any, runoff of silver into the surface water. This use will result in an insignificant contribution to drinking water exposures." (74 FR. at 27451). In asserting that "minimal, if any" runoff will occur as a result of indoor uses of SDC products, EPA agrees that this use pattern and associated label instructions is unlikely to lead to environmental exposures of concern.

As such, there is no viable "mobility pathway" for residues on indoor surfaces to reach the outdoor environment, much less the non-target organisms found there. Furthermore, any "down the drain" off-site transport of silver ion would be rapidly transformed to other less mobile and less toxic forms. This is discussed in more detail below.

Silver is mostly removed by WWTPs

The "minimal runoff" associated with indoor use of SDC products could occur in one of two ways: (1) "down the drain" runoff following off-label use (i.e. using a potable water rinse and disposing of rinsate in the sink); (2) or through land application of wastewater sludge biosolids for fertilization purposes. In the former case, the vast majority of free silver ion (as provided by release from SDC) will be removed during the wastewater treatment process, while in the latter case, the species of silver found in biosolids (silver sulfide) poses very low toxicity to plants due to the extremely strong nature of the covalent bond between the silver and sulfide ions.

In considering the effect of down-the-drain disposal of silver-bearing surficial residues, it is useful to consider a "life cycle" approach to follow the fate and transport of silver ion from its application as an SDC spray into the environment.

This can be split into three areas: (1) pre-treatment (from the home to a wastewater treatment plant); (2) treatment; and (3) post-treatment (transformation in the environment following treatment):

(1) Pre-Treatment – In traveling from home plumbing to the WWTP, there is ample opportunity for positively-charged silver cations to bind to negatively-charged anions, to sorb to organic matter in the water column, or to sorb onto some other reactive surface.

Silver has the strongest binding preference for reduced sulfur species[6] such as sulfates (EPA, 2010), and sulfur in domestic wastewater is both readily available (from urine) and predominantly present as sulfate (Kaegi et al., 2011). Silver ion also bonds to the ample amount of chloride ions present in natural and waste waters that mix in the sewer pipe, forming insoluble silver chloride. These strong bonds result in immobilization in wastewater and surface waters (Adams and Kramer, 1999). In addition to binding to other ligands, silver ions are also attracted to negatively-charged fulvic and humic acids that are found in organic matter (EPA, 2010). Several of the studies cited below demonstrate that the transformation of silver ion into silver chloride and silver sulfate is rapid and occurs prior to the WWTP process.

(2) Treatment – Similar to the opportunities for binding that exist in the waste stream traveling to the WWTP, yet greater opportunities exist at the WWTP itself. Kim et al. (2010) found that silver ion, when bound to sulfur compounds in sludge or other available organic ligands, is much less toxic than free silver ions. Additionally, any silver that sorbs onto available organic matter is highly likely to be removed via agglomeration and precipitation out of the water column (Stensberg et al., 2012).

While the treatment process removes the vast majority of silver[7], it has been reported that a small amount of silver ion can pass through these systems into the aquatic environment (Benn & Westerhoff, 2008). Kaegi et al. (2011) investigated the behavior of silver nanoparticles in a pilot WWTP fed with municipal wastewater and found that most of the silver in both the effluent and sludge was present as silver sulfide and that this reaction occurs rapidly (< 2 hours) under anaerobic conditions. The authors were keen to point out that the rapid transformation time is "considerably shorter than the average hydraulic retention time in the nonaerated tank of the pilot plant (~ 7 h)," implying that there is more than enough residence time in anaerobic tanks of full-scale WWTPs for the "near complete transformation" to silver sulfide. The availability of sulfides (and thus overall sulfidation during the treatment process) was determined by production of sulfide in the anaerobic zones of a sewer biofilm, in an experimental sewer trunk spiked with silver nanoparticles, as reported by Kaegi et al. (2013).

Wang et al. (2012) performed a similar study on a number of nanoscale materials (including silver) for a similar duration using processes and retention times more akin to full-scale WWTP. This work confirmed that nanoscale silver will accumulate in biosolids (rather than effluent) as well as demonstrated that nanoscale silver had "negligible effects on ability of the wastewater

---

[6] According to EPA (2010), the solubility product constant for silver sulfides is on the order of 10^{-49} to 10^{-50}, which is roughly five times less soluble than silver chloride (10^{-10}) and over 15 less soluble than silver acetate (10^{-3}). Thus, even in the presence of both sulfate and chloride ions, silver ion has a much greater preference for sulfate over any other anion.

[7] A mass balance exercise for silver in a WWTP published by Shafer et al. (1998) found that over 95% of the influent silver is removed from the water column and not found in the effluent. Lytle (1984) reported similar removal from WWTPs processing silver-bearing photochemical waste streams. Kaegi et al. (2010) also performed a mass balance calculation on their pilot WWTP data and determined that only about 5% of the silver left the WWTP in effluent – in this case, this amounted to 7.2 grams of silver out of the influent concentration of roughly 130 micrograms/liter that was fed into the pilot system for over 3 weeks. Blaser et al. (2008) modeled the fate and transport of silver ions in the Rhine River and found that the "fraction of silver removed by filtration and treatment" to be 85-99%.

bacteria to biodegrade organic material, as measured by chemical oxygen demand" (Wang et al, 2012). Schafer et al. (2013) reported that silver is eliminated from WWTPs with an efficiency of 95-99% through the rapid transformation (less than 30 minutes) of insoluble silver sulfide mainly attached to flocs of sludge, even when receiving "worst case" influent concentrations of up to 20 μg/L.

Kaegi et al. (2015) reported very low silver concentrations (less than 0.5 μg/L) in the outflow of a WWTP receiving industrial discharge containing silver chloride and silver sulfide nanoparticles, confirming a removal efficiency of greater than 95%. Furthermore, the fraction of silver chloride present in the WWTP influent was almost wholly transformed to silver sulfide in roughly 30 minutes, during sewer transit to the WWTP. There is also recent evidence that the extracellular polymeric substances (EPS), produced by microbes that reside in biofilm and activated sludge, bind silver ion and act as yet another "sink" for any free silver ion that survives the WWTP process. (Geyik et al., 2016).

(3) Post-Treatment – Similar to the pre-treatment water column, there is ample organic matter and reduced sulfur species in all aquatic environments to act to reduce any remaining silver ion in WWTP effluent and continue to reduce bioavailability and toxicity to aquatic plants (Luoma, 2008). In fact, dissolved sulfides, organic materials and chloride ions will bind up "essentially all the free silver ions in fresh waters (making it unavailable for uptake by organisms) and drive the free silver ions to very low levels" (EPA 2010).

Silver ions can form complexes with available chloride and ammonium ions to form soluble complexes, covalent adducts with proteins bearing thiol groups (e.g. glutathione, cysteine) and insoluble salt precipitates (like silver chloride, silver sulfide). (Schafer et al., 2013) Given that silver is "an extremely particle-reactive metal," one would expect silver to be "quickly scavenged from the water column, ending up in sediments" in fairly short periods of time (EPA, 2010).

Most recently, a group of federal researchers conducted a 60-day estuarine mesocosm study using both ionic and nanoscale silver (Cleveland et al., 2012) to simulate movement of silver in a "single-level low marsh." Silver concentrations were then measured via inductively-coupled plasma mass spectroscopy (ICP-MS) in the water column (seawater), sediment, biofilms, hard clams, grass shrimp, mud snails, cordgrass stalks and leaves, and sand. In seawater, silver ion was "completely removed before the first sampling point at 2 h" - which is consistent with the findings of Kaegi et al. (2011) described above. Among the biota (both plants and animals), only mud snails and grass shrimp showed any accumulation of silver in their tissues from the mesocosm ion treatment. Both of these species feed on detritus and organic matter and, thus, uptake of silver into these species was from consumption of food items to which silver ion had sorbed (and not from the water column, as there was no measurable ion in the water column).

Of particular importance is the fact that, in the ion-treated mesocosm, there was no accumulation of silver into biofilms, sediment or sand. The authors speculate that lack of anaerobic conditions at the water-sediment interface or short residence in the water column prevented transfer of silver ion into sediments, as would be expected given the organic matter and ligand content. Overall, the mesocosm study shows very low potential for accumulation in estuarine biota from the water column and that exposure to aquatic organisms would mainly be expected through consumption of silver-bearing food items. In addition, the study confirms the rapid removal of free silver ion from the aquatic environment, thus minimizing the exposure potential to aquatic organisms.

The toxicity of silver to aquatic organisms of regulatory concern is well-characterized

The toxicity and mechanism of silver ions to algae has been well characterized, in both EPA documents and the available open literature. In general, adverse effects of silver ion on aquatic plants "would not be expected under natural environmental conditions because silver can be readily transferred into biologically nonreactive compounds. Sulfides, dissolved and particulate organic matter, chloride, and enzymes within the biota have all been shown to reduce the toxicity of ionic silver" (Ratte, 1999; Levard et al., 2012).

Excellent compilations of the effects of silver on aquatic organisms can be found in:

1. U.S. EPA (1980): Ambient Water Quality Criteria for Silver
2. Eisler (1996): Silver Hazards to Fish, Wildlife, and Invertebrates
3. Ratte (1999): Bioaccumulation and toxicity of silver compounds: a review

Note that these compilations include studies using multiple species of both freshwater and marine fish, as well as mysid shrimp and invertebrates (both freshwater and marine).

U.S. EPA (1980) reports LC50 values for a wide variety of fish, including (see Eisler's Table 6 on pages 26 – 28) mottled sculpin, sheepshead minnow, Atlantic silverside, rainbow trout, fathead minnow, summer flounder, winter flounder and speckled dace. Ratte (1999) has compiled studies done with freshwater organisms, including six species of fish (see Table 3 on page 189), as well as marine organisms, including two species of fish (see Table 4 on page 191). U.S. EPA (1980) reports an LC50 for mysid shrimp *Mysidopsis bahia* of 250 ppb, while Connell et al. (1991) reported a range of LC50 values (2 to 10 ppb) for grass shrimp *Palaemonetes pugio*. U.S. EPA (1980) reports LC50 values for many different invertebrates, including copepod *Acartia tonsa* (36 ppb), daphnid *Daphnia magna* (0.4 to 15 ppb), and mayfly *Ephemerella grandis* (4 to 8.8 ppb). Eisler (1996) reports these studies and many more in Table 6 (see pages 26 – 28). Refer to Ratte's Table 8 (on page 196) for a compilation of studies done with freshwater invertebrates, including effects and threshold concentration data.

Additional studies of value to ascertain the toxicity of silver ion to aquatic organisms of regulatory concern include:

- Hoheisel et al. (2012) exposed *Daphnia magna* and *Pimephales promelas* to acute and sublethal levels of ionic silver, reporting a 96-hr LC50 of 4.70 ug/L and a 7-day EC20 of 1.37 ug/L for Pimephales.

- Hedayati et al. (2012) reported an LC50 of 0.33 ppm for juvenile carp exposed to silver ions (as silver nitrate).

- Lekamge et al. (2018) reported LC50 values for three species of Australian freshwater invertebrates (including *Daphnia*, *Hydra*, and *Paratya* spp) exposed to silver ion (as silver nitrate).

- Ribeiro et al. (2014) exposed *Daphnia magna* and *Danio rerio* to silver nitrate using OECD 202/211 and the OECD fish embryo toxicity tests, respectively. They reported 24- and 48-hour LC50 values and EC50 values for several endpoints for *Daphnia* as well as cumulative mortality and hatching rate data for the fish.

- Volker et al. (2013a) exposed three different species of *Daphnia* to silver nitrate using acute, chronic and multigenerational study designs (i.e. OECD guidelines 202, 211).

- Sakamoto et al. (2014) reported acute and chronic toxicity for three cladocerans (including *Daphnia*) exposed to silver nitrate using OECD guidelines 202 and 211.

- Volker et al. (2013b) exposed freshwater bivalve (*Sphaerium corneum*) to silver nitrate for 28 days to assess reproductive and behavioral toxicity.

- Wang et al. (2012) performed a 48-hour acute toxicity study with silver nitrate and cladoceran *C. sphaericus* as well as a fish early-life stage study using *Danio rerio* embryos.

- Wang et al. (2014) reported on the acute toxicity of silver nitrate to marine organisms (amphipod and mysid species) as well as the bioaccumulation of silver nitrate to marine polychaete worm (using a 28-day marine sediment study).

- Park et al. (2014) reported acute and chronic toxicity data for a freshwater midge (*Chironomus riparius*) exposed to silver nitrate.

It should be noted that there is a substantial body of ecotoxicity literature that investigates the effects of nanoparticulate silver where the reference or comparison treatment is silver ion, often delivered as silver nitrate. This above list was, in part, compiled by a non-exhaustive review of the literature involving exposure of silver ion to aquatic exposures of regulatory concern (in this case: mysids, freshwater and marine invertebrates and sediment-dwelling organisms, and fish).

Finally, there are individual reports examining the reduced toxicity of silver sulfide to aquatic organisms. For example, Hirsch examined the effects of sediments spiked with silver sulfide on a freshwater amphipod (Hirsch, 1998a) and an oligochaete (Hirsch, 1998b). Similar work on amphipods using sediment spiked with silver has been published by EPA (Berry et al., 1996).

Several studies are available that show the reduction of toxicity of silver in the presence of sulfides in *Daphnia* (crustacean) and *Oncorhyncus* (fish) spp. (Bowles et al., 2002; Bianchini et al., 2002; Bianchini et al., 2005; Mann et al., 2004; and Bianchini and Wood, 2008). Again, this review is not exhaustive and it is noted that there are many additional published toxicity reports using nanoparticulate silver sulfide that use silver ion as a control group that are not included here.

As described above, the ecotoxicity of aquatic organisms of regulatory concern such as invertebrates, fish, and mysids has been adequately characterized in the literature and any additional toxicity information collected by the registrant will not improve the existing adequate database. Given that acute and chronic toxicity data is available already for a wide variety of freshwater and marine organisms (including daphnids and other invertebrates, mysid shrimp, sediment-dwelling organisms and fish), the registrant requests waivers for the Mysid chronic toxicity test (850.1350); Fish early-life stage toxicity test (850.1400); Whole sediment: acute freshwater invertebrates (850.1735); Whole sediment: acute marine invertebrates (850.1740); and, 850.1850: Aquatic Food Chain Transfer.

Implications for risk assessment

It is important to note that Risk Assessment and Science Support Branch of the Antimicrobials Division has specifically stated that indoor hard surface applications of SDC product Axen 50 are "anticipated to result in minimal, if any, runoff of silver into groundwater."[8] It is for this reason that AD found a drinking water assessment unwarranted for Axen 50 "based on the uses proposed on this label." Furthermore, the Axen 50 label specifies that "potable water rinse is not permitted following the use of this product as a sanitizer" on non-porous hard surfaces in meat, poultry and dairy plants to prevent runoff at these use sites. In fact, AD considers the subsequent dietary exposures from food processing equipment sanitization so "extremely small that it is considered negligible and not included in the combined or aggregate [dietary] assessments."

The 2009 Final Work Plan for silver and silver compounds specified that an ecological risk assessment would only be conducted for outdoor residential use sites, such as "materials preservative use of siding for housing and stucco; and coatings/films for siding for housing, roofing, shingles, building materials, wood and plastic composites, concrete, cement, and glazes for cement."[9] It was stated in the 2009 summary of environmental fate and ecotoxicity data for silver compounds that the "Agency has an adequate ecological toxicity data base for silver and silver salts. Therefore, these data are adequate for assessing risk from potential ecological exposures to elemental silver and silver salts."[10]

EPA assumptions for "worst-case" residual active ingredient on a surface following application is to assume 1 milligram of SDC solution per square centimeter (cm^2). This would be adjusted for the level of silver ion in that 1 milligram of SDC solution (e.g. up to 0.005%, or 50 ppm silver ion maximum), or 0.005 mg silver ion/cm^2 at worst. As explained above, these small amounts of silver ion would, if released to the environment, encounter the ubiquitous amounts of sulfide and chloride available in the natural world and be readily converted to much more stable silver sulfide or silver

---

[8] Memorandum dated July 21, 2008 from Talia Lindheimer (RASSB/AD) to Marshall Swindell (RMBI/AD). Subject: Revised Dietary, Drinking Water Residential and Occupational Exposure Assessment for the Proposed New Uses of Silver (Axen 50 EPA Reg # 72977).

[9] See the 2009 Registration Review Final Work Plan at www.regulations.gov, Docket EPA-HQ-OPP-2009-0334-0023.

[10] Memorandum dated June 4, 2009 from Najm Shamim (RMBII/AD) to Marshall Swindell (RMBI/AD). Subject: Summary of Product Chemistry, Environmental Fate, and Ecotoxicity Data for Silver, Silver Salts, Silver Zeolites (Copper and Zinc) and Silver Sodium Hydrogen Zirconium Phosphate for Registration Review.

chloride. The presence of sulfur and chloride is not only associated with a decrease of silver ion in the environment but a reduction in toxicity to aquatic animals, including invertebrates, fish and sediment-dwelling organisms.[11] However, it is important to note that "down the drain" exposure could *only* occur through off-label use, such as a potable water rinse following application.

Given the low likelihood of off-site transport of silver ion from indoor use of SDC products and the environmental transformation that silver ion undergoes before, during and after the WWTP process, EPA has previously obviated the need for drinking water and ecological risk assessments for SDC products. Additional data on aquatic organisms would therefore be superfluous.

Conclusions

ETI H2O is respectfully requests waivers for the following studies:

850.1350: Mysid Chronic Toxicity
850.1400: Fish Early-Life Stage Toxicity
850.1735: Whole Sediment: Acute Freshwater Invertebrate Toxicity
850.1740: Whole Sediment: Acute Marine Invertebrate Toxicity
850.1850: Aquatic Food Chain Transfer

ETI H2O is requesting the waivers for the following reasons:

1. The labels for all the SDC products specify indoor uses only. Instructions for use include application to indoor hard surfaces via a non-aerosol-generating-method and do not require wiping the surface or a potable water rinse. Thus, there is no viable mobility pathway for residual silver to travel into the environment, much less come into contact with a non-target organism, aquatic or otherwise.

2. EPA has previously agreed with this assertion in granting SDC products (in a manner that is separate from silver salts and silver zeolites) a tolerance exemption for use on food-contact surfaces. In this approval, EPA recognized that "[t]he uses identified as indoor hard surface applications will result in minimal, if any, runoff of silver into the surface water. The use of silver as a food contact surface sanitizer will result in minimal, if any, runoff of silver into the surface water. This use will result in an insignificant contribution to drinking water exposures." (74 FR 27447, at 27451).

3. This "minimal runoff" actually poses very low exposure potential as the vast majority of silver ions will be removed through the wastewater treatment process. The small amounts of silver ion that may pass through a WWTP unchanged will be removed from the post-treatment water column rapidly and completely, with most of the now non-reactive silver being incorporated into sediment near where it was originally deposited.

[11] Levard, C. et al. (2013). Sulfidation of silver nanoparticles: natural antidote to their toxicity. Env. Sci. Tech. 47(23):13440-8. doi: 10.1021/es403527n. Epub 2013 Nov 15.

4. The toxicity of silver to aquatic organisms (freshwater, marine and estuarine) has already been well characterized by both past EPA documents as well as reports found in the open literature.

5. Given the amount of data available on silver ecotoxicity, additional data would not provide any additional informative value to ecological risk assessment (as wildlife toxicity values for silver already exist). Any data is superfluous anyway as EPA has previously obviated the need for drinking water and ecological risk assessments for SDC products, given their unique nature and labelled uses relative to other silver-based antimicrobial pesticides.

REFERENCES

Adams, N.W.H. and J.R. Kramer. Silver speciation in wastewater effluent, surface waters, and pore waters. Environ. Tox. Chem. 18: 2667-2673, 1999.

Benn, T.M. and P. Westerhoff. Nanoparticle silver released into water from commercially available sock fabrics. Environ. Sci. Technol. 42: 4133-4139, 2008.

Berry, W.J., Hansen, D.J., Mahony, J.D., Robson D.L., etc. Predicting the toxicity of metalspiked laboratory sediments using acid-volatile sulfide and interstitial water normalizations. Environ. Tox. Chem. 15: 2067–2079, 1996.

Bianchini, A., Bowles, K.C., Brauner, C.J., Gorsuch, J.W, Kramer, J.R., and Wood, C.M. 2002. Evaluation of the effect of reactive sulfide on the acute toxicity of silver (I) to Daphnia magna. Part 2. Toxicity results. Environ. Toxicol. Chem. 21, 1294-1300, 2002.

Bianchini, A., Rouleau, C., and Wood, C.M. Silver accumulation in Daphnia magna in the presence of reactive sulphide. Aquat. Toxicol. 72, 339-349, 2005.

Bianchini, A. and Wood, C.M. Do sulfide or water hardness protect against chronic silver toxicity in Daphnia magna? A critical assessment of the acute to chronic toxicity ratio for silver. Ecotoxicol. Environ. Safety. 71, 32-40, 2008.

Blaser, S.A., Scheringer, M., MacLeod, M. and K. Hungerbuhler. Estimation of cumulative aquatic exposure and risk due to silver: contribution of nano-functionalized plastics and textiles. Sci. Total. Environ. 390: 396-409, 2008.

Bowles, K.C., Bianchini, A., Brauner, C.J., Kramer, J.R., and Wood, C.M. Evaluation of the effect of reactive sulfide on the acute toxicity of silver (I) to Daphnia magna. Part 1. Description of the chemical system. Environ. Toxicol. Chem. 21, 1286-1293, 2002.

Cleveland, D., Long, S.E., Pennington, P.L., Cooper, E., Fulton, M.H., Scott, G.I., Brewer, T., Davis, J., Petersen, E.J. and L. Wood. Pilot estuarine mesocosm study on the environmental fate of silver nanomaterials leached from consumer products. Sci. Total Environ. 421-422: 267-272, 2012.

Connell, D., Sanders, J., Riedel, G. and G. Abbe. Pathways of silver uptake and trophic transfer in estuarine organisms. Environ. Sci. Tech. 25: 921–924, 1991.

Diamond, J., Mackler, D., Collins, M. and D. Gruber. Derivation of freshwater silver criteria for the New River, Virginia, using representative species. Environ. Tox. Chem. 9: 1425–1434, 1990.

Eisler, R. Silver Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review. National Biological Service, US Department of the Interior. Biological Report 32. September 1996.

Fabrega, J., Luoma, S.N., Tyler, C.R., Galloway, T.S. and J.R. Lead. Silver nanoaprticles: behavior and effects in the aquatic environment. Environ. Int. 37: 517-531, 2011.

Geyik, A.G. and F. Cehen. Exposure of activated sludge to nanosilver and silver ion: inhibitory effects and binding to the fractions of Extracellular Polymeric Substances. Bioresource Tech. DOI: 10.1016/j.biortech.2016.03.157, 2016.

Hedayati A, Shaluei F, Jahanbakhshi A. Comparison of Toxicity Responses by Water Exposure to Silver Nanoparticles and Silver Salt in Common Carp (Cyprinus carpio). Global Veterinaria 8(2), 179-184, 2012.

Hirsch, M.P. Toxicity of silver sulfide-spiked sediments to the freshwater amphipod (Hyalella Azteca). Environ. Tox. Chem. 17: 601-614, 1998a.

Hirsch, M.P. Bioaccumulation of silver from laboratory-spiked sediments in the oligachaete (Lumbriculus variegatus). Environ. Tox. Chem. 17: 601-614, 1998b.

Hoheisel, S.M., Diamond, S. and Mount, D. Comparison of nanosilver and ionic silver toxicity in Daphnia magna and Pimephales promelas. Environmental Toxicology and Chemistry, 31: 2557-2563. doi:10.1002/etc.1978, 2012.

Kaegi, R., Vogeline, A., Sinnet, B., Zuleeg, S., Hagendorfer, H., Burkhardt, M. and H. Siegrist. Behavior of metallic silver nanoparticles in a pilot wastewater treatment plant. Environ. Sci. Technol. 45: 3902-3908, 2011.

Kaegi, R. et al. Fate and transformation of silver nanoparticles in urban wastewater systems. Water Res. 47: 3866-3877, 2103.

Kaegi, R. et al. Transformation of AgCl nanoparticles in a sewer system – A field study. Sci. Total Env. DOI: 10.1016/j.scitotenv.2014.12.075, 2015.

Kim, B., Park, C.S., Murayama, M. and M.F. Hochella. Discovery and characterization of silver sulfide nanoparticles in final sewage sludge products. Environ. Sci. Technol. 44: 7509-7514, 2010.

Lekamge S, Miranda AF, Abraham A, Li V, Shukla R, Bansal V and Nugegoda D. The Toxicity of Silver Nanoparticles (AgNPs) to Three Freshwater Invertebrates With Different Life Strategies: *Hydra vulgaris, Daphnia carinata*, and *Paratya australiensis*. *Front. Environ. Sci*. 6:152. doi: 10.3389/fenvs.2018.00152, 2018.

Levard, C., Hotze, E.M., Lowry, G.V. and G.E. Brown. Environmental transformations of silver nanoparticles: impact on stability and toxicity. Environ. Sci. Technol. Epub ahead of print, February 29, 2012.

Levard, C. et al. (2013). Sulfidation of silver nanoparticles: natural antidote to their toxicity. Env. Sci. Tech. 47(23):13440-8. doi: 10.1021/es403527n. Epub Nov 15, 2013.

Luoma, S.N. Silver nanotechnologies and the environment: Old problems or new challenges. Project on Emerging Nanotechnologies, 2008.

Lytle, P.E. Fate and speciation of silver in publicly owned treatment works. Environ. Tox. Chem. 3: 21-30. 1984.

Mann, R.M., Ernste, M.J., Bell, R.A., Kramer, J.R., and Wood, C.M. Evaluation of the protective effect of reactive sulfide on the acute toxicity of silver to rainbow trout (Oncorhynchus mykiss). Environ. Toxicol. Chem. 23, 1204-1210, 2004.

Park, S.-Y., Chung, J., Colman, B. P., Matson, C. W., Kim, Y., Lee, B.-C., Kim, P-J., Choi, K., Choi, J. Ecotoxicity of bare and coated silver nanoparticles in the aquatic midge, *Chironomus riparius*. Environmental Toxicology and Chemistry, 34(9), 2023–2032. doi:10.1002/etc.3019, 2015.

Ratte, H.T. Bioaccumulation and toxicity of silver compounds: a review. Environ. Tox. Chem. 18: 89-108, 1999.

Ribeiro, F., Gallego-Urrea, J., Jurkschat, K., Crossley, A., Hassellöv, M., Taylor, C., Soares, A., Loureiro, S. Silver nanoparticles and silver nitrate induce high toxicity to Pseudokirchneriella subcapitata, Daphnia magna and Danio rerio. Science of the Total Environment, 466-467C, 232–241. doi:10.1016/j.scitotenv.2013.06.101, 2013.

Sakamoto, M., Ha, J.-Y., Yoneshima, S., Kataoka, C., Tatsuta, H., & Kashiwada, S. Free Silver Ion as the Main Cause of Acute and Chronic Toxicity of Silver Nanoparticles to Cladocerans. Archives of Environmental Contamination and Toxicology, 68(3), 500–509. doi:10.1007/s00244-014-0091-x, 2014.

Schafer, B. et al. State of the art in human risk assessment of silver compounds in consumer products: a conference report on silver and nanosilver held at the BfR in 2012. Arch. Toxicol. 87: 2249-2262, 2013.

Shafer, M.M., Overdier, J.T. and D.E. Armstrong. Removal, partitioning, and fate of silver and other metals in wastewater treatment plants and effluent-receiving streams. Environ. Tox. Chem. 17: 630-641, 1998.

Stensberg, M.C., Wei, Q., McLamore, E.S., Porterfield, D.M., Wei, A. and M.S. Sepulveda. Toxicological studies on silver nanoparticles: challenges and opportunities in assessment, monitoring and imaging. Nanomedicine (London) 6: 879-898, 2011.

U.S. EPA. Ambient Water Quality Criteria for Silver. 1980. Available at: http://water.epa.gov/scitech/swguidance/standards/criteria/upload/AWQC-for-Silver_1980.pdf

U.S. EPA. Nanomaterial Case Study: Nanoscale Silver in Disinfectant Spray. August 2010. Available at: http://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=498022

Völker C, Boedicker C, Daubenthaler J, Oetken M, Oehlmann J. Comparative toxicity assessment of nanosilver on three Daphnia species in acute, chronic and multi-generation experiments. *PLoS One*. 2013;8(10):e75026. Published 2013 Oct 7. doi:10.1371/journal.pone.0075026, 2013a.

Volker et al. Toxicity of silver nanoparticles and ionic silver: Comparison of adverse effects and potential toxicity mechanisms in the freshwater clam Sphaerium corneum. Nanotoxicology 9(6). https://doi.org/10.3109/17435390.2014.963723, 2013b.

Wang, Y., Westerhoff, P. and K.D. Hristovski. Fate and biological effects of silver, titanium dioxide, and C60 (fullerene) nanomaterials during simulated wastewater treatment processes. J. Hazard. Mater. 201-202: 16-22, 2012.

Wang, Z., Chen, J., Li, X., Shao, J., & Peijnenburg, W. J. G. M. Aquatic toxicity of nanosilver colloids to different trophic organisms: Contributions of particles and free silver ion. Environmental Toxicology and Chemistry, 31(10), 2408–2413. doi:10.1002/etc.1964, 2012.

Wang, H., Ho, K. T., Scheckel, K. G., Wu, F., Cantwell, M. G., Katz, D. R., Burgess, R. M. Toxicity, Bioaccumulation, and Biotransformation of Silver Nanoparticles in Marine Organisms. Environmental Science & Technology, 48(23), 13711–13717. doi:10.1021/es502976y, 2014.